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Impact of ultra-fine milling and air classification on biochemical and techno-functional characteristics of wheat and rye bran

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ABSTRACT

Dry milling and air classification were applied to produce three different ingredients from wheat and rye brans. Dried and pin disc-milled brans having particle size medians of 89–131 μm were air classified to produce protein- and soluble dietary fibre-enriched hybrid ingredients (median particle size 7–9 μm) and additionally brans were ultra-finely milled (median particle size 17–19 μm). The samples were characterised in regard to their composition and techno-functional properties. In air classification, protein content increased from 16.4 and 14.7% to 30.9 and 30.7% for wheat and rye brans, which corresponded to protein separation efficiencies of 18.0 and 26.9%, respectively. Concurrently, the ratio between soluble and insoluble dietary fibre increased from 0.22 to 0.85 for wheat and from 0.56 to 1.75 for rye bran. The protein- and soluble dietary fibre-enriched wheat bran fraction showed improved protein solubility at alkaline pH when compared to pin disc- and ultra-finely-milled wheat bran, whereas less difference between the wheat ingredients was observed at native and acidic pH. The protein- and soluble dietary fibre-enriched rye bran fraction exhibited lower solubility than the pin disc- or ultra-finely-milled rye brans at all the studied pH-values. Ultra-fine milling alone decreased protein solubility and increased damaged starch content when compared to the pin disc-milled brans. Both protein enrichment and ultra-fine milling improved colloidal stability in comparison to the pin disc-milled raw materials. The lowest water and oil binding capacities were obtained for the protein-enriched fractions. Ultrasound-assisted emulsification of the protein- and soluble dietary fibre-enriched fractions and the ultra-finely-milled brans revealed no major differences in the visual quality or stability of the emulsions. The results suggest that modification of the techno-functional properties of cereal brans may be acquired via both air classification and ultra-fine milling.

1. Introduction

Plant-based protein-rich food ingredients are considered sustainable and healthy options when compared to animal-based ingredients (Aiking, 2011; Day, 2013). Especially ingredients from agricultural side-streams such as cereal brans are seen potential considering their positive contribution to resource sufficiency (Nikinmaa et al., 2018; Sozer et al., 2017; Vermeulen et al., 2012) and nutrition as reviewed by Poutanen et al. (2014). Annually, 130 million tons of wheat bran containing approximately 20 million tons of good quality protein and a significant amount of other valuable food components are are lost from the food chain to feed or energy production (FAO, 2019; Prückler et al., 2014; Shiferaw et al., 2013). Production of rye, an important crop in the Nordic countries, yields theoretically annually worldwide in two million tons of bran containing in total approximately 0.3 million tons of protein (FAOSTAT, 2017). Wheat and rye brans are composed of dietary fibre (37–53%), proteins (14–18%) and varying amounts of starch (9–40%) (Kamal-Eldin et al., 2009; Nordlund et al., 2013). Arabinoxylan is the

Abbreviations: DF, dietary fibre; HMWIDF, high molecular weight insoluble dietary fibre; HMWSDF, high molecular weight soluble dietary fibre; LMWSDF, low molecular weight soluble dietary fibre; OBC, oil binding capacity; PDM, pin disc milling; PEF, protein-enriched fraction; PSE, protein separation efficiency; RVA, Rapid Visco Analyser; RyB-PDM, pin disc-milled rye bran; RyB-PEF, rye bran protein-enriched fraction; RyB-UFM, ultra-finely-milled rye bran; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; UFM, ultra-fine milling; WBC, water binding capacity; WhB-PDM, pin disc-milled wheat bran; WhB-PEF, wheat bran protein-enriched fraction; WhB-UFM, ultra-finely-milled wheat bran.

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most abundant dietary fibre, consisting of 19–30% in wheat bran and 16–25% in rye bran (Bataillon et al., 1998; Kamal-Eldin et al., 2009; Nordlund et al., 2012). Other bran dietary fibre components include cellulose, fructan, β-glucan and lignin (Kamal-Eldin et al., 2009).

Osborne classification of wheat bran proteins show that the water-soluble albumins and salt-soluble globulins, that are often co-extracted due to presence of salts in bran matrices, account for 33% of the protein in non-milled (De Brier et al., 2015) and 40% in 0.4 mm sieve-milled wheat bran (Idris et al., 2003). Prolamins constitute for 11% and 18% and glutelins for 7–16% and 26% of the protein in non-milled and sieve-milled wheat brans, respectively, and the share of proteins remaining un-extractable varies between 18 and 49% depending on the particle size of the bran (De Brier et al., 2015; Idris et al., 2003). Due to differences in industrial milling processes applied for wheat bran separation, varying amounts of endosperm and, thus gluten protein, is present in the bran preparations and for example pearling-based debranning, which reduced the presence of endosperm, increased the content of albumins/globulins up to 62–69% (Rizzello et al., 2012). In rye grain, albumins form the main protein class, comprising of 34% of all proteins followed by prolamins (19%), globulins (11%) and glutelins (9%), while 21% of the proteins remain un-extractable (Bushuk, 2001). Literature concerning rye bran proteins remains scarce. As reviewed by Bushuk (2001), the storage proteins in the aleurone cells of the rye grain are globulins and no prolamins are found in the aleurone. In an old study by Rohrlich & Rasmus (1956), albumin and globulin contents of rye bran were determined to be 18.0 and 29.8%, respectively. On the contrary, most of the storage proteins in rye endosperm are prolamins (Bushuk, 2001).

Dry fractionation, including for example air classification, is considered a more energy-efficient and gentle processing approach when compared to wet processing (Schutyser & van der Goot, 2011) and allows production of hybrid ingredients enriched with both protein and fibre (Nikinmaa et al., 2018; Silventoinen et al., 2019). Literature concerning wheat bran protein enrichment by dry fractionation remains little, and relation of fractionation to techno-functional properties has not been reported. Hemery et al. (2011) reached protein content of 19.5% when applying milling and electrostatic separation to wheat bran (16.6% protein). Similarly moderate protein enrichment from 17 to 20% was obtained by electrostatic separation of wheat bran targeting arabinoxylan fractionation (Wang et al., 2015). Protein enrichment to 21.8% in aleurone fraction produced from wheat bran (15.2% protein) (Hemery et al., 2009) was reached using different dry processes. For whole grain wheat flours (9–16% protein), protein enrichment up to 54% was obtained with an extremely low mass yield (0.6%) whereas higher mass yields (11–25%) allowed enrichment up to 21–30% (Wu & Stringfellow, 1992). For rye, sieving-based separation of rye aleurone from whole rye flour allowed protein enrichment from 11.4 to 17.6% (Glitsø & Bach Knudsen, 1999), whereas, to our knowledge, no literature on dry fractionation of rye bran targeting protein enrichment is available.

When assessing the functionality of plant-based protein ingredients, understanding the contribution of associated components present in the ingredient is critical. This applies especially for the dry-fractionated protein ingredients in which the role of the other constituents, such as starch and fibre, in food applicability is evident. Thus far, literature concerning use, technological functionality and applicability of cereal bran-based protein- and fibre-enriched ingredients is limited (Kortekangas et al., 2020; Silventoinen et al., 2019). We have previously reported that dry-fractionated rice bran ingredients show improved techno-functional properties when compared to milled bran as such (Silventoinen et al., 2019). However, research is needed to understand the properties and applicability of the bran-origin protein ingredients from other crops.

The aim of the present work was to investigate the impact of protein enrichment from rye and wheat bran by air classification and particle size reduction by ultra-fine milling of brans on techno-functional properties relevant in liquid food systems. These included protein solubility, colloidal stability, oil and water binding capacities, pasting properties and emulsification.

2. Materials and methods

2.1. Raw materials

Wheat (V6200) and rye (R4500) bran raw materials were commercial samples obtained from Fazer (Fazer Mills, Lahti, Finland).

2.2. Milling and dry fractionation

Prior to pin disc milling both bran raw materials were dried for 48 h at 40 °C in an oven to reach moisture content of 4.9–5.2% (Fig. 1). The dried wheat bran was first milled using a 0.3 mm sieve with an 100 UPZ fine impact mill (Hosokawa Alpine AG, Augsburg, Germany) at a rotor speed of 17800 rpm followed by two times milling with the same apparatus equipped with stainless steel pin disc grinders at a rotor speed of 17800 rpm (WhB-PDM). The dried rye bran was milled twice with an 100 UPZ mill equipped with pin disc grinders (Hosokawa Alpine AG, Augsburg, Germany) at a rotor speed of 17800 rpm (RyB-PDM). These milled samples (WhB-PDM and RyB-PDM) were air classified using a 50ATP classifier (Hosokawa Alpine, Augsburg, Germany) operated at air classifier wheel speed of 15000 rpm and air flow rate of 50 m^3/h to obtain protein-enriched fine fractions WhB-PEF and RyB-PEF, respectively. Mass yields (% dm) of the fine fractions were calculated as (dry weight of fraction) / (dry weight of raw material) × 100%. Protein

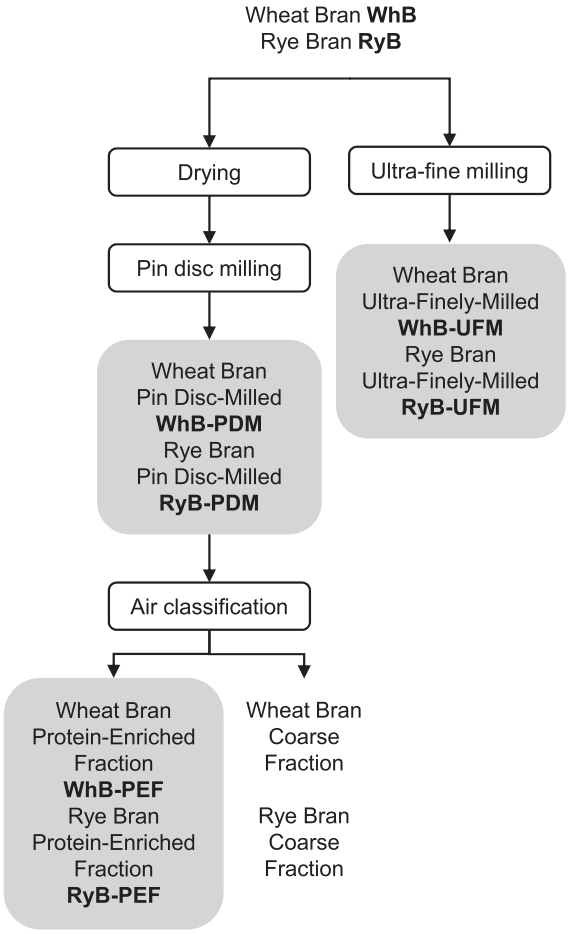

Fig. 1. Schematic presentation of the dry processing and fractionation used for rye and wheat brans.

separation efficiency (PSE % dm) was calculated as (dry weight of fraction × protein content of fraction [% dm]) / (dry weight of raw material × protein content of raw material [% dm]) × 100% (Tyler et al., 1981). All air classifications were performed in duplicate.

In addition, the wheat bran (pre-milled with 0.3 mm sieve) and rye bran (non-pre-milled) were milled without a drying step with Masuko Sanqyo decompression air-flow-type ultra-fine micronizer (Ceren Miller Dau MKCL8-15 J DAU, Masuko Sangyo, Kawaguchi, Japan) using rotor speed of 7600 rpm and trituration time of 1.5 min to obtain ultra-finely-milled wheat (WhB-UFM) and rye (RyB-UFM) bran samples. Ultra-fine milling of bran allowed studying the effect of particle size on the techno-functional properties and comparison of ultra-finely-milled brans and protein-enriched fractions from air classification with rather similar particle sizes.

2.3. Particle size

The particle size distribution of the bran samples was analysed from suspensions by laser diffraction with Beckman Coulter LS 230 (Beckman Coulter Inc., CA, USA) as described in Silventoinen et al. (2019).

2.4. Biochemical composition

Protein content was calculated based on total nitrogen content (N × 6.25) determined by Kjeldahl method according to the AOAC method 2001.11 using an autoanalyser (Thiex, Manson, Andersson, & Persson, 2002). Total starch content was quantified according to the AACC 76–13.01 method using Megazyme total starch assay kit. Damaged starch content was measured according to AACC 76–31.01 method with Megazyme starch damage assay kit. The method was modified slightly as a sample blank absorbance value measured once for each sample was subtracted from the absorbance value of the actual sample in order to exclude the absorbance caused by free glucose. High molecular weight insoluble dietary fibre (HMWIDF), high molecular weight soluble dietary fibre (HMWSDF) and low molecular weight soluble dietary fibre (LMWSDF) were analysed using the enzymatic–gravimetric AOAC method 2011.25 according to McCleary et al. (2012). Ash content was quantified gravimetrically after combustion at 550 °C. The phytic acid content was measured according to the method described by Latta & Eskin (1980) and modified by Vaintraub & Lapteva (1988) using phytic acid dodecasodium salt from corn (P-8810, Sigma) as a standard. All biochemical analyses were performed in duplicate.

2.5. Protein profile

Molecular weight distribution of the proteins in raw material brans (WhB-PDM and RyB-PDM) and air classified fractions (WhB-PEF and RyB-PEF) was visualized by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) under reducing conditions as described in Silventoinen et al. (2019). Each well was loaded with the same amount of protein (30 μg). The protein bands were visualised with Criterion Stain Free Imager and examined using Image Lab software (Bio-Rad).

2.6. Techno-functional properties

Protein solubility (%) analysis was carried out as described in Silventoinen et al. (2019). In brief, the samples were hydrated in water at 2% protein concentration and pH was left unadjusted (6.7 ± 0.2) or adjusted to 5 or 8. The pH was readjusted at 30 and 60 min after which the supernatants were separated by centrifugation (10000*g*, 15 min, 20 °C). Amount of protein released in the supernatant was determined by Kjeldahl method (N × 6.25). Water binding capacity (WBC) was analysed by mixing samples (1 g) with distilled water (10 ml), incubating for 30 min (vortexing every 10 min) and defined as the amount of water (g) retained by the sample (g) under centrifugation (2000*g*, 10 min) (Quinn & Paton, 1979). Oil binding capacity (OBC) was analysed by dispersing samples (100 mg) with sunflower oil (1 g). After 30 min incubation (vortexing every 10 min), the supernatant was removed by centrifugation (3000*g*, 10 min). OBC was defined as the amount of oil (g) retained per solid (g) as defined by Lin et al. (1974). The pasting properties were determined with The Rapid Visco Analyser (RVA) (Newport Scientific Pty Ltd., Warriewood, Australia) with the standard Newport Scientific Method 1 (STD1) using total sample size of 28.5 g and dry matter content of 12.28%. Colloidal stability was analysed by visual observation of the sedimentation of a 4% w/w ingredient dispersion, prepared under magnetic mixing for 30 min, as a function of time. Protein solubility was analysed twice from two replicate extractions. WBC and OBC were analysed as triplicates. RVA analysis was performed twice. In the colloidal stability test, one analysis of duplicate dispersions was performed.

Emulsification of WhB-UFM, RyB-UFM, WhB-PEF and RyB-PEF was assessed by adding 10% rapeseed oil to 10% w/w dispersions. Additionally, a control dispersion (10% w/w, without oil) was prepared to confirm that the observed colloidal stability of the emulsified system was not due to the use of ultrasound treatment as such which showed great impact on dispersion stability (without oil) but rather due to emulsification taking place. Emulsions were prepared with ultrasound using a VC 750 ultrasonic processor (Sonics & Materials, Inc., Newtown, CT, USA) equipped with a stainless steel probe (13 mm in diameter) and operated at 20 kHz using an amplitude of 70% for 3 min. The treatment was performed for 75 ml of dispersion placed in an 150 ml flat-bottomed cylinder (54 mm in diameter), which was immersed in an iced water bath preventing samples from overheating and allowing to retain the temperature <37 °C. The ultrasound probe was placed halfway up the liquid level. Fresh ultrasonicated samples were poured into glass tubes for visual observation of emulsion stability during 1d. Particle sizes of the emulsions were analysed in filtered Milli-Q water with a Mastersizer 3000 Hydro (Malvern Analytical, Worcestershire, UK) after 30 min and 1d of the ultrasonication using refractive indices of 1.33 and 1.53 for media and samples, respectively.

2.7. Statistical analysis

Statistical analysis of the protein solubility, WBC and OBC values was performed using SPSS Statistics software (version 26, IBM, Armonk, NY, USA). For protein solubility, two replicate results from each of the two replicate extractions, and for WBC and OBC three replicate results were analysed by one-way analysis of variance (ANOVA). The level of significance was set at $p < 0.05$ and was assessed by Tukey's post hoc test.

3. Results and discussion

3.1. Particle size reduction

Effect of pin disc milling, ultra-fine milling and air classification on particle size distribution of wheat and rye brans was evaluated to assess the impact of particle size on biochemical and techno-functional ingredient characteristics. Moreover, particle size is a key factor determining the flour behaviour in air classification (Teunou et al., 1999). Pin disc milling of the pre-dried brans resulted in median particle sizes of 131 and 89 μm for wheat (WhB-PDM) and rye (RyB-PDM) brans, respectively, and both brans exhibited wide particle size distributions where the sizes ranged from approximately 3 to 1000 μm (Fig. 2). Air classification of the dried and pin disc-milled bran samples led to fine protein-enriched fractions with median particle sizes of 9 and 7 μm for wheat (WhB-PEF) and rye (RyB-PEF), respectively. Despite the monomodal and rather narrow particle size distributions, both brans also showed presence of some larger particles (approximately up to 210 for WhB-PEF and 50 μm for RyB-PEF) (Fig. 2). Ultra-fine milling of the non-pre-dried brans allowed particle size reduction up to median particle sizes of 19 μm for wheat (WhB-UFM) and 17 μm for rye (RyB-UFM). Like

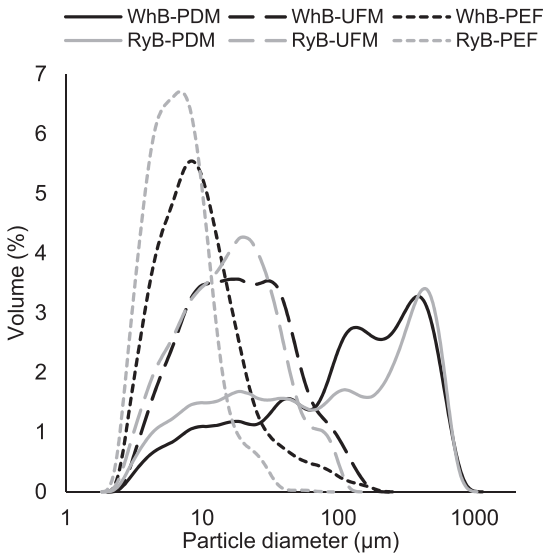

Fig. 2. Particle size distributions of the pin disc-milled wheat and rye brans (WhB-PDM and RyB-PDM, respectively), ultra-finely-milled wheat and rye brans (WhB-UFM and RyB-UFM, respectively) and protein-enriched fractions produced by air classification from wheat and rye brans (WhB-PEF and RyB-PEF, respectively).

in the case of the protein-enriched fractions, also the ultra-finely-milled brans exhibited monomodal but wider particle size distributions ranging from 3 to 210 μm (WhB-UFM) and to 150 μm (RyB-UFM) (Fig. 2).

Structural disintegration of insoluble bran polysaccharides is a prerequisite for liberating protein from the bran aleurone layer cells both by wet and dry means (Arte et al., 2016; Hemery et al., 2011) thus, pin disc milling was performed prior to bran air classification aiming at protein enrichment. However, bran materials, especially wheat bran, are known to be relatively resistant to particle size reduction by dry processing (Hemery et al., 2011; Rosa-Sibakov et al., 2015). To enhance milling, the brans were dried (final moisture content of 4.9–5.2%) prior to pin disc milling, which led to smaller particle size after milling and allowed higher mass yields of the protein-enriched fractions during air classification without compromising the protein content when compared to fractionation of the non-dried material (data not shown), which has been previously shown for field pea and faba bean (Tyler & Panchuk, 1982). On the other hand, too efficient particle size reduction can impair protein fractionation in air classification. For example, we have previously shown that pre-milling results in enrichment of fibrous cell wall structures into the fine, protein-enriched fraction of defatted rice bran, thus lowering the protein content (Silventoinen et al., 2019). In addition, fragmentation of friable pericarp layer may occur in ultra-fine milling, as reported for wheat bran by Antoine et al. (2004) and might result in insoluble dietary fibre fractionation to the protein-enriched fraction again lowering protein content. Moreover, formation and enrichment of damaged starch may occur, as reported in air classification of peas (Pelgrom et al., 2013) and wheat (Wu & Stringfellow, 1992) and jet milling of rye (Drakos, Kyriakakis, et al., 2017). Formation of damaged starch was also observed during ultra-fine milling in this study (Table 1), and therefore air classifications were only performed for the pin disc-milled raw materials (WhB-PDM and RyB-PDM). Interestingly, ultra-fine milling also caused a slight increase in total dietary fibre content (Table 1). Modification of IDF to SDF has been reported as a result of high-intensity ball milling of wheat bran (Van Craeyveld et al., 2009) and potentially explains the increased HMWSDF content in this study. However, concurrent decrease in HMWIDF content would have been expected and reasoning for the unaffected HMWIDF content may lie in formation of new polymer interactions (Van Craeyveld et al., 2009) but requires further research to be confirmed.

Table 1
Mass yield and chemical composition of the pin disc-milled wheat bran (WhB-PDM), ultra-finely-milled wheat bran (WhB-UFM), protein-enriched wheat bran fraction produced by air classification (WhB-PEF), pin disc-milled rye bran (RyB-PDM), ultra-finely-milled rye bran (RyB-UFM) and protein-enriched rye bran fraction produced by air classification (RyB-PEF). PSE, protein separation efficiency; DF, dietary fibre; HMWIDF, high molecular weight insoluble dietary fibre; HMWSDF, high molecular weight soluble dietary fibre; LMWSDF, low molecular weight soluble dietary fibre.

	WhB-PDM	WhB-UFM	WhB-PEF	RyB-PDM	RyB-UFM	RyB-PEF
Mass yield (% dm)[a]	100	100	9.6 ± 0.7	100	100	12.9 ± 0.3
Protein content (% dm)[b]	16.4 ± 0.3	na	30.9 ± 0.7	14.7 ± 0.1	na	30.7 ± 0.5
PSE (% dm)[a]	100	100	18.0 ± 1.0	100	100	26.9 ± 1.1
Starch (% dm)[a]	15.9 ± 0.3	na	14.2 ± 0.0	40.1 ± 0.4	na	36.3 ± 1.3
Damaged starch (% dm) (% of starch)[b]	2.0 ± 0.0 (12.5)	3.4 ± 0.0 (21.5)	3.6 ± 0.2 (25.1)	2.2 ± 0.0 (5.6)	5.0 ± 0.1 (12.5)	4.2 ± 0.1 (11.4)
DF (% dm)[a]	51.7 ± 1.7	56.3 ± 0.4	23.0 ± 1.2	33.1 ± 1.3	38.2 ± 1.1	15.1 ± 0.6
HMWIDF (% dm)[a]	42.4 ± 0.4	42.8 ± 0.1	12.4 ± 0.4	21.3 ± 0.5	23.5 ± 0.0	5.6 ± 1.1
HMWSDF (% dm)[a]	5.0 ± 0.9	9.4 ± 0.3	5.6 ± 0.5	6.3 ± 1.3	9.6 ± 1.0	3.4 ± 0.5
LMWSDF (% dm)[a]	4.2 ± 0.4	4.1 ± 0.2	5.0 ± 0.2	5.5 ± 0.5	5.0 ± 0.0	6.0 ± 0.0
Ash (% dm)[a]	6.0 ± 0.0	na	14.8 ± 0.0	4.0 ± 0.1	na	6.6 ± 0.1
Phytic acid (% dm)[a]	4.7 ± 0.1	na	13.6 ± 0.2	2.1 ± 0.0	na	4.0 ± 0.1

na, not analysed.
[a] ± average deviation.
[b] ± standard deviation.

3.2. Component fractionation in air classification

Air classification of WhB-PDM and RyB-PDM allowed protein-enrichment from 16.4 and 14.7% to 30.9 and 30.7% into WhB-PEF and RyB-PEF, respectively (Table 1). Mass yields of WhB-PEF and RyB-PEF were 9.6 and 12.9%, resulting in protein separation efficiencies (PSE) of 18.0 and 26.9%, respectively. The higher mass yield of RyB-PEF presumably resulted from the overall smaller particle size of RyB-PDM than WhB-PDM, which allowed a higher share of material to enter to the fine fraction. Several different factors for conversion of nitrogen to protein content have been used for cereal ingredients. In this study we decided to use the value 6.25 recommended by FAO (2003) since specific factors for both wheat and rye bran were not available, and as the main purpose of the study was to analyse fractionation of protein, not to compare absolute values between rye and wheat. However, it has been shown that the factors may vary for different cereal crops and fractions of grains (reviewed by Mariotti et al., 2008), and thus, for future studies especially for comparing absolute values between wheat bran, rye bran and their fractions, specific conversion factors for each of these ingredients should be determined.

Protein fractionation by air classification was also evident in terms of protein composition, as analysed by reducing SDS-PAGE (Fig. 3). Enrichment of the proteins sizing 10, 17–18, just below 25 and 32 kDa to the fine wheat bran fraction (WhB-PEF) was observed, suggesting successful concentration of aleurone origin albumin and globulin proteins that are known to have molecular weights between 14 and 60 kDa (De Brier et al., 2015; Schalk et al., 2017). On the other hand, share of the potentially albumin/globulin origin proteins at around 14, 20 and 25 kDa was decreased in the fine WhB-PEF fraction during air classification. In general, proteins sizing 17, 22, 25, 27, 29, 32 and 40 kDa are reported to be present in albumin/globulin fraction (De Brier et al., 2015) and were also detected in the current study (Fig. 3). Regarding other protein

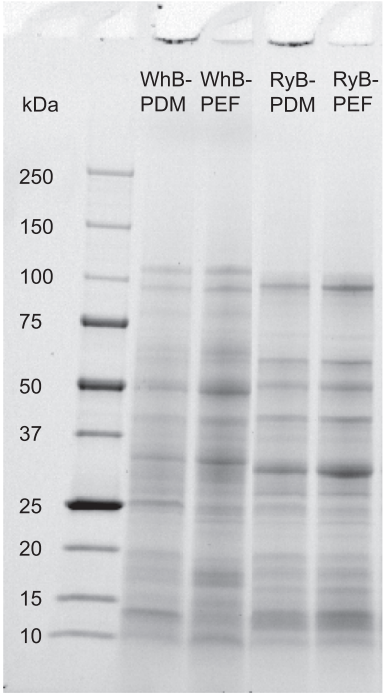

Fig. 3. SDS-PAGE of the pin disc-milled wheat and rye brans (WhB-PDM and RyB-PDM, respectively) and protein-enriched fine fractions produced by air classification from wheat and rye brans (WhB-PEF and RyB-PEF, respectively) under reducing conditions.

classes, De Brier et al. (2015) reported that especially the presence of wheat prolamins having molecular weight of 30–45 kDa (α-type and γ-type gliadins) and 66 kDa (ω-type gliadin) (Lagrain et al., 2012) in bran reveals impurities deriving from the endosperm. On the contrary, releasing entrapped protein from aleurone cells increases quantity of glutelin having molecular weights of 80–120 kDa (HMW glutenins) and 32–45 kDa (LMW glutenins) (Lagrain et al., 2012), thus, suggesting potential origination of glutelins also from the aleurone and not necessarily from the endosperm. Thus, in the present study, defining the origin of the higher molecular weight gluten-related proteins remains challenging. Additionally, identification of the proteins sizing 32–45 kDa as well as protein at 50 kDa, which shows clear enrichment to WhB-PEF, remains challenging since those may derive from aleurone or endosperm glutelins (glutenins), endosperm prolamins (gliadins) or even from albumin/globulin fraction of the aleurone (De Brier et al., 2015).

In rye bran samples, the proteins with sizes of 12–14, 30, 40, 50, 55 and 100 kDa were somewhat enriched in RyB-PEF and all protein bands of the RyB-PDM were also visible in RyB-PEF (Fig. 3). Of those enriched, proteins at 12–14 kDa most probably represent albumins (Redant et al., 2017), which in rye have molecular weights below 66 kDa, more specifically 55 kDa, 40–45 kDa, 25–30 kDa and 15 kDa when visualized under reducing conditions. Rye globulins are detected at around 32 kDa and at 35–60 kDa (Redant et al., 2017) and secalins at 50 and 100 kDa (Schalk et al., 2017). Thus, protein bands of 30, 40 and 55 kDa may originate from either albumins, globulins or secalins. It must be noted, however, that specific analysis of rye bran proteins by SDS-PAGE is missing from literature. For both wheat and rye bran samples, the intensity of the large protein aggregates or particles remaining in the loading wells of the gel was more pronounced for the WhB-PDM and RyB-PDM when compared to WhB-PEF and RyB-PEF.

Regards to carbohydrate composition, initial starch content of WhB-PDM (15.9%) was clearly lower than that of RyB-PDM (40.1%) (Table 1), which most probably results from differences in milling procedures during bran production. Contrary to protein fractionation, no clear impact of air classification on starch content was observed for either of the brans as only minor reductions to 14.2 and 36.3% were observed. On the other hand, enrichment of damaged starch to the protein-enriched fraction took place during air classification for both wheat and rye samples. The share of damaged starch from total starch increased from 12.5 to 25.1% and from 5.6 to 11.4% for wheat and rye bran, respectively. In both cereals, starch is located in larger A-type granules (up to 37–40 and 48 μm for wheat and rye, respectively) and smaller B-type granules (up to 10 and 12 μm for wheat and rye, respectively) in the endosperm and subaleurone (Heneen & Brismar, 1987). Thus, fractionation of the smaller starch granules as well as damaged starch together with the protein, which is known to be located in the small-sized protein bodies/aleurone grains (Pernollet, 1978), may explain the even distribution of starch in fractionation. On the other hand, an evident fractionation of dietary fibre components took place during air classification. For wheat bran, the content of high molecular weight insoluble dietary fibre (HWMIDF) was reduced from 42.4% in WhB-PDM to 12.4% in WhB-PEF and for rye bran, contents of both HMWIDF and HMWSDF reduced from 21.3 and 6.3% (RyB-PDM) to 5.6 and 3.4% (RyB-PEF), respectively. In wheat the amounts of both high (HMWSDF) and low (LMWSDF) molecular weight soluble dietary fibres and in rye the amount of LMWSDF remained rather unaffected. These changes resulted in increases in the soluble-to-insoluble dietary fibre ratios from 0.22 to 0.85 for wheat and from 0.56 to 1.75 for rye bran. Presence of lower amounts of insoluble dietary fibre in the protein-enriched fractions suggest removal of insoluble cellulose and lignin originating from the pericarp structures like we have previously reported also for air-classified, protein-enriched rice bran (Silventoinen et al., 2019).

Phytic acid contents of WhB-PDM and RyB-PDM were 4.7 and 2.1%, which are well in line with the values of 3.5–4.2 and 1.4–3.8% reported for wheat and rye brans, respectively (Kamal-Eldin et al., 2009). In air classification, phytic acid contents increased to 13.6 and 4.0% in WhB-PEF and RyB-PEF, respectively. The distribution of phytic acid was studied since we have previously reported it to enrich drastically to the protein-enriched fraction produced by air classification from defatted rice bran (Silventoinen et al., 2019) and to affect fraction functionality (Kortekangas et al., 2020). In the current study, phytic acid fractionation also suggests that aleurone protein, which is stored as globoids that are known to contain protein and phytic acid (Bohn et al., 2007) and have even been used as markers for successful aleurone fractionation from wheat bran (Antoine et al., 2004) is enriched in the protein fraction. In addition to phytic acid enrichment, the protein-enriched fractions had higher ash contents than the raw material brans. This enrichment supposedly resulted partly from enrichment of phytic acid, which contains phosphorus and binds minerals, all of which are analysed as ash in the composition. Both wheat and rye aleurones are known to be enriched with protein and ash, which further allows us to assume enrichment of aleurone layer to the WhB-PEF and RyB-PEF (Bucsella et al., 2016; Glitsø & Bach Knudsen, 1999).

3.3. Techno-functional properties of the ingredients

Protein solubility is considered a key prerequisite for other techno-functional properties, such as gelation, emulsification and foaming, and when the bran samples' protein solubility in water was analysed, significant differences between different pH-values, fractions and raw materials were revealed. For all the ingredients, solubility was the lowest at pH 5 and the highest at pH 8. At pH 5, protein solubility of PEF < UFM < PDM for both wheat and rye and the same trend was observed for rye at native pH and pH 8. On the contrary, for wheat the protein solubility of UFM < PDM < PEF at the other studied pH values (native and pH 8). In addition, alkaline pH induced a more pronounced increase in solubility of wheat than rye bran proteins. The UFM and PDM materials differed only in terms of milling intensity, and ultra-fine milling decreased protein solubility compared to pin disc milling at all pH values

and for both raw materials. This could presumably be due to the impact of harsher milling conditions, e.g. heat generation, which may alter protein properties or even cause polymer interactions and aggregation (Van Craeyveld et al., 2009). Indeed, similar ultra-fine milling of rapeseed press cake proteins has been earlier shown to result in partial protein denaturation (Rommi et al., 2015).

Previous studies have reported lower protein solubility values for wheat bran proteins. For example, Idris et al. (2003) reported wheat bran protein solubility of 14% at pH 5.5 and De Brier et al. (2015) observed solubility of 12–14% at pH-values 5.5–6.5. Similarly, solubility at pH 7.5 was only 18% according to De Brier et al. (2015) who utilised non-milled wheat bran in their studies. On the other hand, increased solubility of 40%, more similar to what was obtained in the present study (WhB-PDM 43.9% at native pH and 65.6% at pH 8), was observed at pH 7.5 by Idris et al. (2003) who examined bran that was ground using a mill equipped with a 0.4 mm sieve. In the current study, a 0.3 mm sieve was applied and milling was further continued with a more impactful pin disc mill, which suggests that the more efficient milling presumably improved protein solubility but was not too harsh to result in reduced protein solubility as observed after ultra-fine milling in this study. Significant changes in the solubility of especially albumin/globulin proteins due to particle size reduction have been also reported by De Brier et al. (2015) who studied both non-milled and ball-milled wheat bran and linked the increased solubility with disruption of aleurone cell wall structures during intensive milling (Van Craeyveld et al., 2009). Since, endosperm derived gluten has limited solubility (<10%) at pH range 6–8 and only slightly improved solubility at pH 5 (23%) (Deng et al., 2016), the more pronounced impact of alkaline pH on improving protein solubility of WhB-PEF when compared to non-fractionated WhB-PDM or WhB-UFM suggest successful enrichment of soluble aleurone proteins during air classification, as was also observed by SDS-PAGE (Fig. 3).

On the contrary, limited research is available on the rye bran and aleurone protein composition. Nordlund et al. (2013) studied protein digestibility of native rye bran and reported protein solubility of 31.9% in sodium phosphate buffer (20 mM, pH 6.9, containing 10 mM NaCl) which is in the same range as 42.2% observed for RyB-PDM (in water at native pH 6.7) in this study. The impact of rye flour particle size on aqueous protein extraction was investigated by Drakos et al. (2017b). They applied alkaline extraction of rye proteins followed by acidic precipitation and observed that jet milled rye flour proteins were less efficiently extracted than proteins from the normal flour, similarly as was noticed in the current study between RyB-PDM and RyB-UFM. The reduced solubility was suggested to result from potential particle aggregation that would hinder solvent diffusion to the matrix (Drakos, Kyriakakis, et al., 2017; Drakos, Malindretou, et al., 2017). The solubility decrease observed in air classification of rye bran is potentially due to the presence of insoluble proteins inside aleurone cells or formation of aggregates during pin disc milling that hinder further protein liberation even from the smallest particles in the protein-enriched fraction. However, verifying these hypotheses should be a subject for a following study entity.

Water and oil binding capacity of bran samples was analysed as part of the techno-functionality evaluation in order to predict their food applicability. Ingredients with high OBC may be applied for stabilising emulsions or high-fat food systems as reviewed in Elleuch et al. (2011). High WBC, on the other hand, can be considered as an advantage when increased viscosity or thickening properties are desired but may impair the technological functionality of ingredients if functionality forming components compete for the available water (Katina et al., 2006; Kinsella, 1976). WhB-PEF and RyB-PEF showed the lowest WBC values (1.2 g/g) among all the samples (Table 2). Interestingly, for wheat, the highest value was obtained for WhB-PDM (2.7 g/g) and WhB-UFM had lower value of (2.2 g/g) whereas for rye, RyB-UFM exhibited the highest value (1.9 g/g) followed by RyB-PDM (1.5 g/g). For rye, the higher WBC of RyB-UFM when compared to RyB-PDM presumably results from the increased amount of damaged starch which is known to have improved water binding capacity when compared to native starch as starch becomes more prone to hydration and exhibits higher surface area (Berton et al., 2002; Drakos, Kyriakakis, et al., 2017; Pelgrom et al., 2013). However, the same was not detected for the wheat bran samples potentially due to the lower damaged starch content in WhB-UFM (3.4%) than in RyB-UFM (5.0%) or due to the changes occurring in the insoluble dietary fibre structures as the dietary fibre content was much higher in the wheat than rye bran samples. Brans (PDM and UFM) contain larger amounts of insoluble dietary fibre than the protein-enriched fractions (PEF), which may explain the differences in the WBC between those samples as insoluble arabinoxylan (Berton et al., 2002; Courtin & Delcour, 2002) and cellulose are known to exhibit high WBC. As reviewed by Elleuch et al. (2011), grinding of DF ingredients may either increase the WBC due to increased surface area or decrease WBC as a result of damaging the structures responsible for the water binding, and different grinding methods may also affect the binding differently. Results supporting the fact that smaller particles often exhibit lower WBC, analysed by standard centrifugation method, are reported for coarse wheat bran after particle size reduction from 900 to 320 μm (Auffret et al., 1994) and also after more intensive size reduction from coarse wheat bran to 262 and further to 30 μm (De Bondt et al., 2020), latter of which is close to the particle sizes of the current raw materials. PEF samples exhibited the lowest WBC values most probably due to small particle size of the fine fractions and partly due to compositional differences. OBC was only mildly affected by the different dry processes and raw materials as values of 1.0–1.4 g/g were obtained for all the samples (Table 2). However, for both wheat and rye samples it appeared that the protein-enriched fractions (WhB-PEF and RyB-PEF) had the lowest values and pin disc-milled raw materials (WhB-PDM and RyB-PDM) the highest. This is in accordance with the study of Drakos et al. (2017a) where smaller particle size resulted in lower OBC which the authors attributed to reduced amount of oil getting physically entrapped.

Pasting properties of the bran ingredients were studied to evaluate their behaviour during heating. Peak and final viscosity values varied

Table 2
Protein solubility at pH 5, native, and 8, and water (WBC) and oil (OBC) binding capacities as well as peak and final viscosities from RVA of the pin disc-milled wheat and rye brans (WhB-PDM and RyB-PDM, respectively), ultra-finely-milled wheat and rye brans (WhB-UFM and RyB-UFM, respectively) and protein-enriched fractions produced by air classification from wheat and rye brans (WhB-PEF and RyB-PEF, respectively).

	Protein solubility (%)			WBC (g/g)	OBC (g/g)	Peak viscosity (cP)	Final viscosity (cP)
pH	5	native	8	native	native	native	native
WhB-PDM	38.5 ± 0.6^{c}	43.9 ± 0.3^{d}	65.6 ± 0.5^{f}	2.7 ± 0.04^{c}	1.3 ± 0.01^{c}	158 ± 0	222 ± 1
WhB-UFM	34.3 ± 0.7^{b}	38.2 ± 0.4^{c}	53.8 ± 0.8^{e}	2.2 ± 0.03^{b}	1.1 ± 0.02^{b}	146 ± 0	280 ± 1
WhB-PEF	30.1 ± 1.0^{a}	45.1 ± 0.5^{d}	75.5 ± 0.5^{g}	1.2 ± 0.04^{a}	1.0 ± 0.01^{a}	92 ± 2	167 ± 7
RyB-PDM	38.4 ± 1.2^{cd}	42.2 ± 0.5^{e}	50.5 ± 0.2^{g}	1.5 ± 0.01^{b}	1.4 ± 0.00^{c}	496 ± 1	580 ± 6
RyB-UFM	36.6 ± 0.4^{bc}	40.2 ± 1.0^{d}	47.2 ± 1.0^{f}	1.9 ± 0.01^{c}	1.1 ± 0.01^{b}	789 ± 5	1032 ± 3
RyB-PEF	31.9 ± 1.2^{a}	34.9 ± 0.6^{b}	43.6 ± 0.1^{e}	1.2 ± 0.07^{a}	1.0 ± 0.02^{a}	427 ± 1	598 ± 4

Results with different letters, separately for the two raw materials, and for solubility, WBC and OBC, are significantly ($p < 0.05$) different from each other.

greatly between wheat and rye samples as rye bran ingredients showed more than two times higher values than the corresponding wheat bran ingredients (Table 2). This most probably resulted from the dramatic differences in starch contents of wheat (14.2–15.9%) and rye (36.3–40.1%) bran ingredients as higher starch content in general results in higher viscosity, but this is not solely dependent on concentration but amylose/amylopectin ratio as well. Protein-enriched wheat bran, most probably enriched with the small B-starch granules, showed lower viscosity values than the raw material WhB-PDM which is in line with the findings of Kumar & Khatkar (2017) who reported lower peak and final viscosity values for B-type wheat starch granules than for the non-fractionated wheat starch. The same applied also for peak viscosities of RyB-PEF and RyB-PDM whereas final viscosity values showed the opposite trend. Moreover, addition of insoluble dietary fibre is known to contribute to elevated peak and final viscosities of rice starch suspensions (Lai et al., 2011) and was also in line with the peak viscosity values of PDM and PEF samples of both brans and final viscosity values of WhB-PDM and WhB-PEF. Interestingly, WhB-UFM and WhB-PDM samples did not differ remarkably regarding the viscosity values whereas considerable increases were seen in viscosities of RyB-UFM (789 and 1032 cP) when compared to RyB-PDM (496 and 580 cP). This is in contrast with the results reported by Hasjim et al. (2013) who observed positive correlation between the final viscosity and increased particle size and negative correlation between the final viscosity and amount of damaged starch in rice flour.

Dispersability and colloidal stability of food powders are important characteristics for the stability of medium and high moisture food applications. Colloidal stability of the water dispersions of the ingredients at native pH showed positive correlation with decreased particle size (Fig. 4). Both pin disc-milled materials sedimented already after 5 min of standing. On the contrary, WhB-UFM and RyB-UFM started to sediment after 10 min of standing and somewhat similar behaviour was also observed for RyB-PEF. Interestingly, WhB-PEF remained stable, apart from minor clarification from the top of the sample all through the 30 min observation time. This fraction exhibited the highest protein solubility at native pH, which may have improved the colloidal stability as well. Several authors have reported improved colloidal stabilities for cereal bran samples with reduced particle size (De Bondt et al., 2020; Rosa-Sibakov et al., 2015; Silventoinen et al., 2019). In the current study the larger median particle size of the ultra-finely milled wheat bran sample (19 μm) compared to the protein-enriched wheat bran fraction (9 μm) may possibly explain the lower stability of the WhB-UFM compared to WhB-PEF. Contrary to WhB-samples, the larger median particle size of RyB-UFM than RyB-PEF did not result in more pronounced sedimentation probably owing to differences in compositions of rye and wheat bran samples. Moreover, it must be noted that not only size, but also particle density and fraction of soluble components that affect continuous phase viscosity play a role in dispersion stability.

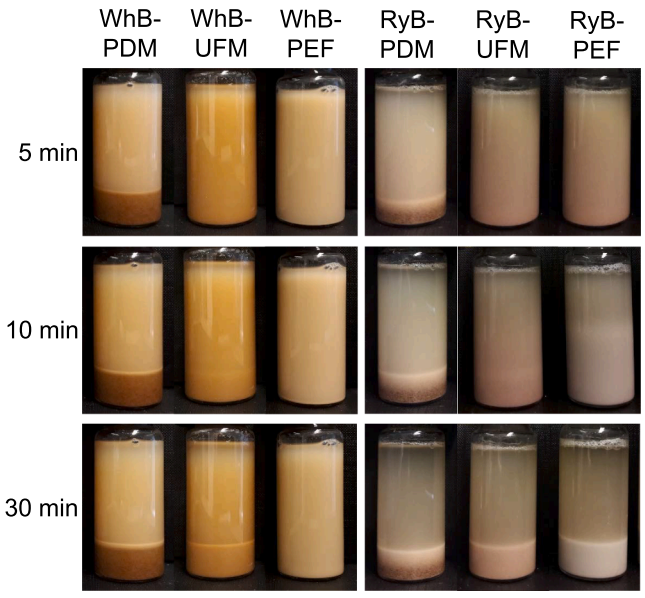

Fig. 4. Colloidal stability of the pin disc-milled wheat and rye brans (WhB-PDM and RyB-PDM, respectively), ultra-finely-milled wheat and rye brans (WhB-UFM and RyB-UFM, respectively) and protein-enriched fractions produced by air classification from wheat and rye brans (WhB-PEF and RyB-PEF, respectively) at 4% dry matter content after 5, 10 and 30 min of standing.

Emulsification of the rye and wheat bran ingredients was investigated to obtain evidence about their applicability in emulsified food systems, such as plant-based milk substitutes. Based on the results from the colloidal stability analysis (Fig. 4), emulsification was carried out only for the most stable raw material ingredients (UFM) and fractions (PEF). Emulsions were prepared using ultrasound treatment which has recently proven as an advantageous emulsification method (Cabrera-Trujillo et al., 2018; Gaikwad & Pandit, 2008). Both PEF and UFM samples of wheat and rye brans formed emulsions that were visually homogeneous and stable almost 1 day and showed only minor clarification on top (Fig. 5). Control samples were prepared without oil addition in order to identify the effect of ultrasonication on stability as is. Emulsions created by ultrasonication formed clearly more stable dispersions compared to the case where ultrasonication without oil addition was performed (control samples). Oil droplets were efficiently stabilized by the surface active molecules or particles present within the multi-component ingredients forming an emulsified system. The nature of the emulsions, however, was not elucidated within this work. The fresh emulsions naturally exhibited higher viscosity when compared with the control samples ultrasonicated without oil (data not shown) which also contributed to physical stability of the emulsion in comparison to powder dispersion. However, ultrasonication of 10% powder without oil addition was studied as a control system to emulsions solely to show that this process alone increased the dispersion characteristics of the fractions, when compared to dispersions prepared using magnetic mixing only shown in Fig. 4.

The particle size distribution of the emulsions remained unaffected over time apart from RyB-PEF sample which coarsened slightly during 1d. (Fig. 6). Moreover, based on microscopy, ultrasonication did not alter starch structure as starch granules show birefringence in the form of maltese cross under polarized light (Supplementary Fig. S1). Thorough understanding of the factors affecting properties of emulsions prepared from the current multicomponent systems requires evaluation of the role of both proteins but also dietary fibre components which may have impact on the emulsion stability. As reviewed by Dickinson (2013) and McClements (2007), food emulsions are often stabilised by both surface active emulsifiers and components modifying the properties, such as viscosity, of the continuous phase. In addition, solid particles may play a role in a specific emulsion type, referred to as a Pickering emulsion (Dickinson, 2013). Research on the role of bran proteins in emulsions remains scarce and both negative results showing poor emulsion properties (Arte et al., 2019) and positive results about creation of stable emulsions (Chandi & Sogi, 2007; Idris et al., 2003) have been reported for wheat and rice bran proteins. In regard to polysaccharides, the bran materials are not supposed to contain considerable amounts of polysaccharides having interfacial activity such as gum arabic, pectin and galactomannan (Costa et al., 2019), whereas soluble arabinoxylans may contribute to emulsion stability by increasing the viscosity of the continuous aqueous phase or by adsorbing to the oil-water interface as has been studied with purified soluble arabinoxylans from wheat and rye flours (Mikkonen et al., 2008).

4. Conclusions

Incorporation of protein and fibre containing ingredients deriving from agricultural side-streams into liquid food systems has been

Fig. 5. Stability of emulsions (10% dry matter content mixed with 10% rapeseed oil) and control dispersions (10% dry matter, no oil) prepared from wheat and rye bran raw materials milled with ultra-fine mill (WhB-UFM and RyB-UFM, respectively) and protein-enriched fractions produced by air classification (WhB-PEF and RyB-PEF, respectively) after 30 min, 2 h and 1 d of standing.

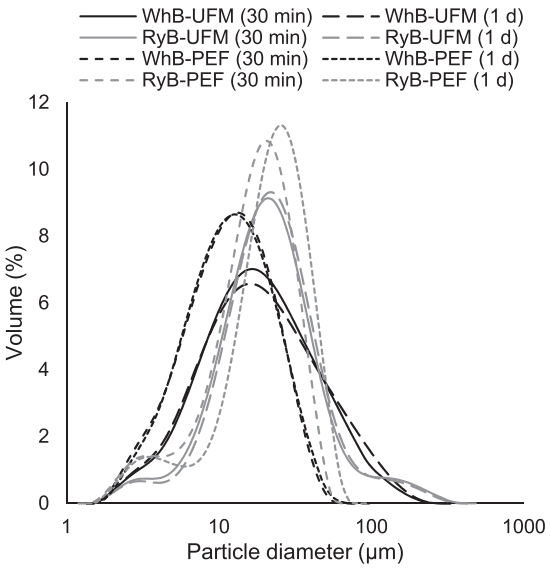

Fig. 6. Particle size distributions of emulsions (10% dry matter content mixed with 10% rapeseed oil) prepared from wheat and rye bran raw materials milled with ultra-fine mill (WhB-UFM and RyB-UFM, respectively) and protein-enriched fractions produced by air classification (WhB-PEF and RyB-PEF, respectively) after 30 min and 1d of standing.

considered challenging due to for example low solubility, instability and coarse mouthfeel. In the current work, we demonstrated that the techno-functional properties of cereal brans may be altered either by protein enrichment or ultra-fine milling. However, the two processing methods affect the functionalities differently and the choice of the processing method should be based on different ingredient requirements for specific target food applications. Air classification of dried and pin disc-milled wheat and rye brans allowed protein enrichment from 15 and 16% to 31% and increased the ratio of soluble to insoluble DF in the fine protein-enriched fraction. Based on protein composition analysis and enrichment of phytic acid, air classification most probably allowed enrichment of aleurone proteins into the protein fraction. Production of protein-enriched fraction by air classification resulted in increased colloidal stability for both brans and improved protein solubility for wheat bran, whereas the opposite effect on rye bran protein solubility was observed. Compared to pin disc milling, ultra-fine milling improved colloidal stability, decreased protein solubility and resulted in formation of damaged starch, which affected water binding capacity of the ingredients. The results infer that depending on the milling intensity, particle size reduction may have either positive or negative impact on bran protein solubilisation. However, both protein-enriched and ultra-finely milled ingredients also allowed production of stable emulsions which suggests improved food applicability.

CRediT authorship contribution statement

Pia Silventoinen: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Anni Kortekangas:** Conceptualization, Validation, Investigation, Data curation, Writing - review & editing, Visualization. **Dilek Ercili-Cura:** Methodology, Writing - review & editing, Supervision. **Emilia Nordlund:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109971.

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